Bioavailability of Dissolved Organic Nitrogen in Wastewater Effluent as Determined by Resin Separation

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ABSTRACT

Dissolved organic nitrogen (DON) typically accounts for less than 10% of the nitrogen in effluent from conventional wastewater treatment plants. However, biological nutrient removal (BNR) systems are capable of achieving very low concentrations of inorganic nitrogen species. As a result, DON can account for the majority of the total nitrogen in the effluent of BNR treatment plants and that not all of the effluent DON stimulates algal growth in receiving waters. Available data suggest that it is difficult to remove the DON from the effluent of BNR treatment plants. To develop a better understanding of the occurrence and bioavailability of effluent DON, a new protocol was developed for measuring the bioavailable DON and inert DON (iDON) in effluents from BNR treatment plants. The method employed an XAD-8 resin to separate hydrophobic forms of DON from hydrophilic forms and an anion exchange resin to remove nitrate from the hydrophilic DON fractions. To assess the bioavailability of wastewater-derived DON from the hydrophilic and hydrophobic fractions, algal growth assays were performed in the presence of bacteria in effluents from four municipal wastewater treatment plants. The results showed that the hydrophobic DON did not stimulate algal growth, that there was little difference in algal growth and DON consumption between the untreated and XAD-8 treated samples, and that 10-29% of the effluent DON (i.e., 0.1-0.3 mg N/L) was inert. This resin separation technique can be used to quantify and separate iDON, bioavailable DON and nitrate.

KEYWORDS

Humic substances, algae, eutrophication, bioassay, biological nutrient removal

INTRODUCTION

Effluent from municipal wastewater treatment plants equipped with biological nutrient removal (BNR) systems contains inorganic and organic forms of nitrogen. Organic nitrogen can be divided into dissolved and particle-associated forms, as defined by the fraction passing through a 0.45 μm filter. In most cases, nitrate is the predominant form of inorganic nitrogen, with concentrations up to 20 mg N/L. In effluent from many BNR treatment plants, concentrations of nitrate range are often below 3 mg N/L (Pagilla et al., 2006). These systems normally include effluent filtration or membrane separation processes capable of achieving particle-associated nitrogen concentrations less than 0.1 mg N/L. Concentrations of dissolved organic nitrogen (DON) in these plants typically range from 0.5 to 2 mg/L (Pagilla et al. (2006; Westgatet and Park 2010; Pehlivanoglu-Mantas and Sedlak, 2006). Thus, DON can account for a significant fraction of the total nitrogen discharged by BNR treatment plants.

Historically, water quality models and regulations designed to control eutrophication have considered all DON to be available for uptake by algae. While this approach was convenient when DON represented a small fraction of the nitrogen released to surface waters, it complicates current efforts to control eutrophication in watersheds where BNR treatment plants are being operated. If a portion of the effluent DON is not readily bioavailable to organisms in receiving waters, efforts to further reduce eutrophication by removing DON could be less effective than predicted by water quality models. Furthermore, allocating resources to control inert forms of DON that do not cause eutrophication results in unnecessary consumption of energy and natural resources and slows efforts to control more important nitrogen sources.

Previous research suggests that a portion of effluent DON is not bioavailable to bacteria and algae. For example, Pehlivanoglu and Sedlak (2004) reported that approximately half of the DON in denitrified wastewater effluent was not available to nitrogen-limited algae when bacteria were present to facilitate the breakdown of the organic matter. Similar results have been reported by other researchers using laboratory bioassays to assess DON uptake by algae (Urgun-Demirtas et al., 2008; Sattayatewa et al., 2009). However, additional research is required before these results can be extrapolated to other locations because previous measurements of DON were either limited to wastewater effluent from plants with extremely low concentrations of nitrate or the methods used to measure DON had considerable uncertainty due to the effects of relatively high nitrate background concentrations. Furthermore, attempts to identify the source of this inert DON (iDON) have provided little insight into the factors affecting its treatment or environmental fate.

This research project, which was part of WERF Nutrient Challenge, was conducted to provide a method for measuring bioavailable DON and iDON in wastewater effluent. The approach used for nitrogen separation and quantification employs equipment that is available in most utility and commercial laboratories. The algal bioassay uses a common species of algae and incubation conditions that are routinely used in the assessment of nutrient uptake and toxicity testing.

METHODOLOGY

Separation of hydrophobic organic compounds was accomplished by passing samples through an Amberlite XAD-8 resin (Rohm and Haas). For most samples, a total of 5.45 grams of resin (wet density = 1.09 g/mL, 1BV = 5 mL) was placed in a FlexColumn (10 mm ID; 100 mm length, Kontes) combined with flow adapter (Kontes) to minimize the dead volume.

To separate hydrophobic and hydrophilic forms of DON, effluent samples were acidified to pH 2 with HCl. The acidified sample was passed through the XAD-8 resin at 0.2 bed volumes per minute. The water passing through the column contained the hydrophilic forms of DON. To elute the hydrophobic forms of DON from the column, a 0.1 N solution of NaOH was passed through the column at a rate of 0.2 bed volumes per minute from reverse direction.

Nitrate was removed from the hydrophilic fraction of the effluent DON with a Dowex 1X8 anion exchange resin (Fluka, Cl^--form, Strong basic resin). In most applications, 1 gram of resin was packed into a 7-mm diameter, 150-mm long Kontes glass column. Effluent samples were pulled through the sample at a fixed rate with a peristaltic pump, attached to the column with Teflon tubing.

Details of resin pre-treatment and method development studies will be provided in the final project report.

Samples were analyzed for nitrogen species using the methods described by Pehlivanoglu and Sedlak (2004). Concentrations of dissolved organic carbon (DOC) were measured using a carbon analyzer (Shimadzu TOC-5000).

Algal bioassays were conducted on the four samples using methods described previously (Pehlivanoglu and Sedlak, 2004). Briefly, the bioassay technique involves the growth of *Selanastrum capricornutum* in whole or fractionated wastewater effluent samples to which all necessary nutrients other than nitrogen have been added. In addition, an inoculum of bacteria, collected from the biological wastewater treatment plant where the effluent sample was obtained, was added to enhance the release of labile forms of organic nitrogen from the DON. Control bioassays employed deionized water (negative control) and 0.7 mg N/L of nitrate added to the hydrophobic fraction (positive control). For both controls, inoculum and algae were added prior to incubation. A small amount of algal growth typically was observed in the controls due to the presence of bioavailable forms of nitrogen in the inoculum.

For the algal bioassay, the untreated sample was incubated immediately after addition of nutrients, bacteria and algae. The pH of the hydrophilic sample was adjusted to 7.0 with NaOH prior to addition of nutrients, algae and bacteria. For the hydrophobic sample, the DON was concentrated by approximately a factor of four because the volume of NaOH used to elute the sample was less than the original sample volume. The pH of the eluted sample was adjusted to 7.0 with HCl prior to initiation of the algal bioassay experiment.

All treatments were conducted in triplicate. Samples were analyzed at 0,2,4,7, 10 and 14 days for DON, DOC and nitrate using methods described in section 2.2.3. Chlorophyll a was measured by fluorescence as described previously (Pehlivanoglu and Sedlak, 2004).

RESULTS

Wastewater effluent samples used in this study spanned a range of total nitrogen concentrations. Nitrate concentrations ranged from <0.1 mg N/L at the Truckee Meadows Water Reclamation Facility, which fully denitrifies, to 9.7 mg N/L at the San Jose/Santa Clara Water Pollution Control Facility (Table 1). Effluent DON ranged between 0.66 and 1.01 mg N/L and was not correlated with nitrate concentrations. Hydrophobic DON accounted for 10 to 29% of the DON.

Table 1 – Concentrations (mg N/L) of Nitrogen Species in wastewater effluent samples

Facility	Nitrate	DON	Hydrophobic DON
Truckee	<0.1	1.01±0.02	0.29±0.02
King William	0.2±0.1	0.66±0.04	0.14±0.01
Broad Run	5.8±0.1	1.01±0.23	0.11±0.01
San Jose[1]	9.7±0.1	0.94±0.64	0.23±0.02

[1] Effluent samples also contained 0.1 mg N/L of ammonium. Ammonium concentrations were <0.1 mg N/L in all other samples.

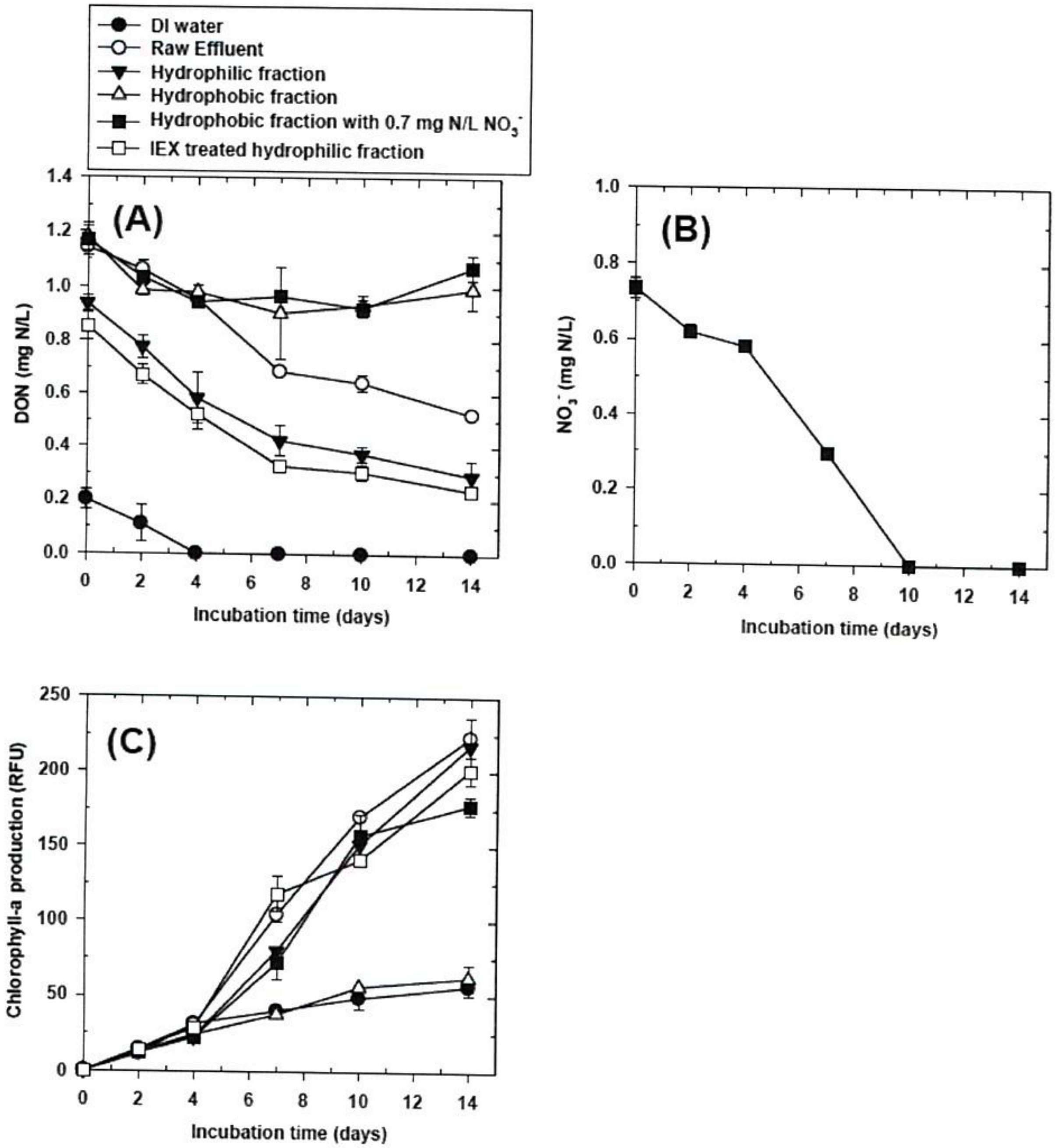
Results from the bioassay experiments conducted in effluent from the Truckee Meadows Wastewater Treatment Plant (Figure 1) indicate substantial differences between the hydrophilic and hydrophobic forms of DON. Concentrations of hydrophilic DON (shaded triangles) decreased during the incubation as chlorophyll a concentrations increased whereas concentrations of hydrophobic DON (open triangles) remained constant (after accounting for loss of DON added with the bacterial inoculum). Chlorophyll a in the hydrophobic samples did not increase beyond what was observed in the deionized water control (shaded circles).

A small amount of hydrophilic DON was lost during passage through the anion exchange columns (compare open squares to shaded triangles). For the Truckee sample, the concentration of hydrophilic DON only decreased by approximately 5% during resin treatment. For samples from the other three treatment plants, loss of hydrophilic ranged from 15 to 31%. While these losses complicated assessment of the bioavailability of hydrophilic forms of DON, it had no impact on assessment of the bioavailability of hydrophobic DON.

Overall, chlorophyll a production in the untreated and hydrophilic fractions was roughly comparable to that observed in the hydrophobic fraction amended with 0.7 mg N/L of nitrate (filled squares). This finding is consistent with our hypothesis that the hydrophilic DON is bioavailable. These results also demonstrate that the sample treatment did not impede the ability of the algae to grow when nitrate was available.

Similar results were obtained when the experiments were repeated with effluent samples from the other three treatment plants. For samples from the Broad Run and San Jose treatment plants, where significant concentrations of nitrate were present, it was difficult to compare the effect of hydrophilic DON on algal growth before and after anion exchange treatment because growth due to nitrate was much greater than growth from hydrophilic DON.

Figure 1 – Concentrations of DON (A), nitrate (B) and chlorophyll a (C) during incubation of an effluent sample from the Truckee Meadows Water Reclamation Facility



DISCUSSION

Results from these experiments suggest that hydrophilic DON is capable of stimulating algal growth while the hydrophobic DON is not available to algae. In three of the four samples, chlorophyll a production during a 2-week incubation of concentrated hydrophobic DON extracts was not different from deionized water controls. In all four samples, concentrations of hydrophobic DON remained constant throughout the 2-week period. In contrast, hydrophilic DON, which had been passed through an anion exchange resin to remove nitrate, stimulated significant algal growth, relative to controls. Chlorophyll a production from nitrate-amended hydrophilic fractions suggested that nearly all DON was available to the algae. Concentrations of hydrophilic DON also decreased during incubation indicating the hydrophilic DON was not inert. However, a substantial fraction of the hydrophilic DON remained at the end of the incubation, which is consistent with production of DON by growing algae and bacteria.

Our findings suggest that a relatively simple test, namely passage of an acidified effluent sample through a hydrophobic resin, can be used to determine the fraction of DON that is incapable of stimulating algal growth under the conditions used in a standard algal bioassay. Data from four wastewater treatment plants indicated that the concentration of hydrophobic DON ranged from 0.11 to 0.29 mg N/L and that hydrophobic DON accounted for 10 to 29% of the DON. Additional research is needed to determine if this relatively narrow range of hydrophobic DON concentrations occur in other wastewater treatment plants.

The conditions used in these experiments may not fully represent the potential for transformation of DON in surface waters. In effluent-receiving surface waters, ultraviolet light, more diverse microbial communities and variations in ionic strength could result in additional transformation of DON (Bronk et al., 2010). Research is needed to assess the potential for transformation of hydrophobic DON under conditions that better mimic those encountered in surface waters.

Finally, the observation that inert DON exhibits hydrophobic characteristics suggests that iDON may be derived from humic substances present in the source water or humic-like substances in sewage or formed during wastewater treatment. This finding also raises the possibility that inert forms of phosphorus in wastewater effluent also may be associated with hydrophobic organic matter. Bioassays conducted by exposing phosphorus-limited algae to hydrophobic and hydrophilic fractions from wastewater effluent could be used to assess this possibility.

CONCLUSIONS

Wastewater effluent samples contain hydrophobic and hydrophilic forms of DON. In this study, the hydrophobic forms of DON, which accounted for less than 30% of the DON, were stable during a 2-week incubation in the presence of algae and bacteria. In contrast, concentrations of hydrophilic forms of DON decreased during incubations. The decrease in hydrophilic DON concentrations was accompanied by an increase in algal growth. These findings suggest that hydrophobic DON is inert (i.e., the DON extracted on the XAD-8 resin is iDON) and hydrophilic DON is available for uptake by algae, provided that bacteria are present to facilitate its transformation.

The DON fractionation method will be useful to researchers and operators of wastewater treatment plants interested in quantifying iDON. This approach may also be useful to researchers interested in sources of inert organic phosphorus. Additional research is underway to assess the resin separation approach on samples from wastewater treatment plants employing a range of BNR treatment technologies.

ACKNOWLEDGMENTS

The project team would like to acknowledge and thank JB Neethling for his advice and support. We are particularly grateful to Randall Gray of the City of Reno, Charles Bott from the Hampton Roads Sanitation District and Deborah Bronk from the Virginia Institute of Marine Science as well as the staff of the Truckee Meadows, San Jose/Santa Clara, Broad Run and Hampton Roads Wastewater Treatment Plants wastewater treatment plants for their cooperation in obtaining samples. This research was sponsored by the Water Environment Research Foundation (WERF) as part of the Nutrient Challenge.

REFERENCES

Pagilla, K.R., Urgun-Demirtas, M. and Ramani, R. (2006) Low effluent nutrient technologies for wastewater treatment. *Water Science & Technology* 53(3), 165-172.

Westgatet, P.J. and Park, C. (2010) Evaluation of Proteins and Organic Nitrogen in Wastewater Treatment Effluents. *Environmental Science & Technology* 44(14), 5352-5357.

Pehlivanoglu-Mantas, E. and Sedlak, D.L. (2006) Wastewater-derived dissolved organic nitrogen: Analytical methods, characterization, and effects - A review. *Critical Reviews In Environmental Science & Technology* 36(3), 261-285.

Pehlivanoglu, E. and Sedlak, D.L. (2004) Bioavailability of wastewater-derived organic nitrogen to the alga Selenastrum Capricornutum. *Water Research* 38(14-15), 3189-3196.

Urgun-Demirtas, M., Sattayatewa, C. and Pagilla, K.R. (2008) Bioavailability of dissolved organic nitrogen in treated effluents. *Water Environment Research* 80(5), 397-406.

Sattayatewa, C., Pagilla, K., Pitt, P., Selock, K. and Bruton, T. (2009) Organic nitrogen transformations in a 4-stage Bardenpho nitrogen removal plant and bioavailability/biodegradability of effluent DON. *Water Research* 43(18), 4507-4516.

Bronk, D.A., Roberts, Q.N., Sanderson, M.P., Canuel, E.A., Hatcher, P.G., Mesfioui, R., Filippino, K.C., Mulholland, M.R. and Love, N.G. Effluent Organic Nitrogen (EON): Bioavailability and Photochemical and Salinity-Mediated Release. *Environmental Science & Technology* 44(15), 5830-5835.